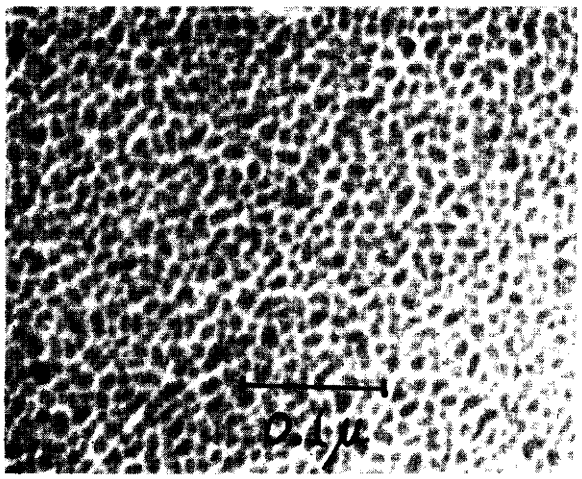

FIG. 6. Same region of the nitrocellulose film as in Fig. 5, after 5 min in the electron beam of the microscope.

ping configuration, the latter becoming less the higher the degree of asymmetry of the substrate.

During the preparation of the substrate films the molecules are generally in a tangled state, and in order to draw them out into a linear structure it is necessary to produce local stresses. This occurs naturally during the drying of the films, and it is only necessary to hunt for regions that have been strained in order to find the desired linear effect. Fig. 4 shows what appears to be a mapping of the molecules in an unstrained state with the polymers lying in a tangled or skein-like configuration.

Figs. 5 and 6 are of the identical portion of a gold deposit on a nitrocellulose film, the former being exposed as soon as a focus was obtained, and the latter exposure made 5 min later, the object remaining in place in the electron microscope and subjected to the 300-μa electron beam between pictures. It will be noted that, whereas Fig. 5 shows most of the particles connected with others, Fig. 6 shows that the metal formerly lying in the connecting filaments has, under the excitation of the electron bombardment, migrated to the larger metallic groups, with the result that the particles have drawn themselves into more stable configurations.

This phenomenon clearly supports the mechanism discussed above as to the formation of these structures. The method is not "shadowing" in the sense that this term is employed in the preparation of transparent objects for electron microscopy; because of the migration of the metal atoms after they arrive on the substrate the structure seen in the present manner is another order of magnitude smaller.

Although an obvious step toward improvement of the resolution of this process would seem to lie in applying Levinstein's finding that metals having higher melting points will form smaller particles, it happens that some metals, palladium and nickel in particular, form continuous films in such thin deposits that the mapping is not striking. Thus for the configuration of the metallic deposit to be responsive to the details of the substrate, it is necessary that the metal used be one which shows a distinct tendency toward migration.

It might be mentioned that the present procedure is a close analog to the conventional staining of specimens for observation under the light microscope; in both instances the contrast between object and background is increased by the selective absorption or attraction of some material having a high stopping power for the radiation being used.

References

1. LEVINSTEIN, H. *J. Applied Phys.*, **20**, 306 (1949).
2. SENNETT, R. S., and SCOTT, G. D. *J. Optical Soc. Am.*, **40**, 203 (1950).
3. LENNARD-JONES, J. E. *Trans. Faraday Soc.*, **28**, 333 (1932); *Proc. Phys. Soc. (London)*, **49**, 140 (1937).

Peptide Structure and Denaturation of Proteins

A. R. Goldfarb

Department of Biochemistry,
Chicago Medical School, Chicago, Illinois

The denaturation of proteins has been described in terms of altered solubility, appearance of functional groups, and change of shape of the molecule (*1*). It has been reported from this laboratory that absorption spectra data at 205 mμ can be correlated with the peptide structure of proteins. It was hypothesized that any alteration of configuration of the protein might have an influence on the absorption due to the peptide bond by changing the electron configuration of this bond. In a series of preliminary experiments very dilute solutions of bovine plasma albumin were heated to 100° C, and immediately chilled at given intervals. The optical densities of these solutions were determined. At the concentrations used and under the conditions of the experiments, no turbidity was formed and the solutions were optically clear. The optical density rose markedly to values which depended on the concentration of protein (9–40 mg/l) and on the time of heating. After the initial rise the density showed a gradual fall with continued heating. This type of curve is qualitatively confirmed, but the quantitative confirmation is not very good. The maximum rate of rise in density occurs in a matter of seconds, and it is conceivable that the quenching of the initial rise has not been reproducible under the conditions of these preliminary experiments.

During this early work it was also observed that if a concentrated solution of the protein (about 1%) was rapidly heated to a boil and immediately cooled, a precipitate formed during the heating. This precipitate on dilution dissolved to an optically clear solution. The precipitate was more soluble in water at pH 3.5 and 7.5. If, however, the suspension was heated for 5 min or longer, the precipitate was completely insoluble both on dilution and by alteration of pH and was soluble only at pH about 10.

It would appear from this preliminary data that

denaturation may involve several steps that are either competitive or successive. In general the steps involved would be (*a*) an alteration of the steric relation between the constituent amino acids so as to alter the peptide bond configuration, (*b*) a reaction in which an equilibrium spatial configuration is reached that is consistent with the temperature and time of heating, (*c*) an interaction of proteins (precipitation) in more concentrated solutions, which is competitive with reaction (*a*). This step is indicated by the finding that the redissolved precipitates show very nearly the same density as the undenatured proteins. In this case it is presumed that the number of peptide bonds altered at the time of precipitation of the protein is sufficiently small so that no change in density is detectable, (*d*) a continued change of the precipitated protein leading to greater insolubility. It is not possible to discuss this last step in terms of the absorption characteristics at this time, since solutions of the precipitate at high pH cannot be studied at present.

This study is being continued further, and the effect of other types of denaturing agents will be investigated, as well as the kinetics of the change.

Reference

1. NEURATH, H., *et al. Chem. Revs.*, **34**, 157 (1944).

A Method for Embedding Undecalcified Bone for Histologic Sectioning, and its Application to Radioautography

James S. Arnold[1]

Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Illinois

In developing a high resolution radioautographic method for the study of the distribution of radioisotopes in bone it is highly desirable to obtain thin sections of bone. Up to the present, the method of choice has consisted of embedding in celloidin and covering the cut surface of the block with an additional thin coat of celloidin prior to sectioning (*1–3*).

Celloidin embedding has proved satisfactory for comparatively soft bones, such as those of young rats, mice, and rachitic animals, and the bone of osteochondral junctions. However, celloidin[2] lacks the adhesive and cohesive properties necessary to hold individual particles of brittle bone firmly in the section. Furthermore, this embedding medium insufficiently impregnates the interior of dense cortical bone. Thus, when harder material is embedded in celloidin, crumbling, breaking, and distortion occur, making the sections unsuitable for autographic or histologic use.

To improve the adhesive and cohesive properties of the embedding medium, the effect of the addition of various plasticizers—Herculin D, castor oil, and diamyl phthalate—was tested. Diamyl phthalate evidenced the best properties for this purpose. Penetration of the embedding medium was increased by (1) using acetone as the solvent (rather than 50% ether-alcohol, and (2) embedding under partial vacuum, followed by higher temperature (60° C) and pressure (3 atm).

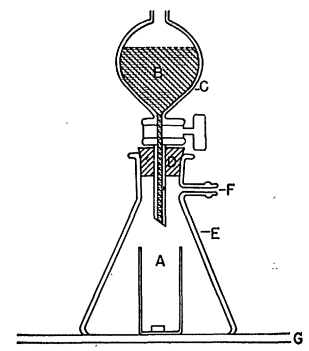

FIG. 1. Vacuum embedding apparatus. *A*, Vial containing tissue; *B*, solution of plastic; *C*, separatory funnel; *D*, rubber stopper; *E*, suction flask; *F*, outlet to water aspirator; *G*, plate glass and ground upper surface.

After the problem of obtaining sufficiently thin sections of bone is solved, preparation of radioautographs is comparatively simple. In this work, a modification of the methods independently developed by Endicott and Yagoda (*4*) and T. C. Evans (*5*) was used. By our method, the sections were mounted on slides that had been coated with a nuclear track emulsion. All studies during the developmental period were made on bones that contained varying amounts of plutonium.[3]

Tissues may be fixed in any desired fixative for routine histologic purposes. When radioautographs are to be made, however, the possibility of the fixative leaching out or redistributing the contained isotopes or producing chemical blacking of nuclear track emulsion on contact must be taken into account. Absolute acetone was found to be a satisfactory fixative, since plutonium is not leached from the tissues. Following fixation, tissues not fixed in acetone are progressed into absolute acetone which has been dried with sodium sulfate. This serves the dual purpose of dehydration and preparing the tissues for embedding in acetone solution of plastic. The plastic itself is prepared as follows: 11 parts air-dried ½-sec nitrocellulose is mixed with 9 parts diamyl or dibutyl phthalate and dissolved in anhydrous acetone so that the final solution contains 50–60% solids by weight. Previously prepared tissue is removed from the absolute acetone and placed in a vacuum embedding chamber. This chamber consists of a separatory funnel in the top of a vacuum flask with the bottom cut out, the flask resting on a lubricated, ground surface of plate glass

[1] The author wishes to acknowledge the assistance of Atlee S. Tracy, who prepared the photomicrograph.

[2] Celloidin is nitrocellulose dissolved in 50% ether, 50% alcohol. In use, it is evaporated to the proper hardness and stored in 70% alcohol. Under these circumstances alcohol and water act as plasticizing materials, separating the molecules of nitrocellulose and altering its hardness.

[3] Plutonium was used because of its uncomplicated α-decay and the convenience of working with low-background emulsions suitable for α-track recording.